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A NOTE ON THE USE OF EVANS BLUE AS A BACKGROUND STAIN IN THE FLUORESCENT TREPONEMAL ANTIBODY TEST*

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Treponeme suspensions for use in the Fluorescent Treponemal Antibody (FTA) test are usually prepared in the protein-containing basal medium used in the Treponemal Immobilization (TPI) test. Such suspensions sometimes show fluorescent staining of debris in the background which may make it difficult to assess the intensity of fluorescence shown by the treponemes, especially with tests on sera showing borderline reactivity. It was thought that, if the background could be stained a constrasting colour to the apple-green fluorescence of the treponemes, the reading of results might be made easier. The use of Evans blue as described by Nichols and McComb (1962) with trachoma and related antigens was investigated. This dye fluoresces red under ultra-violet light and thus affords a good contrast to the specific green fluorescence.

Materials and Methods

The technique used differed from that of Deacon, Freeman, and Harris (1960) in that a slide rotator was not used and the 1 in 200 dilutions of sera, in phosphate buffered saline, pH 7·3, were left in contact with the treponemes for 1 hour at 35°C. Anti-human gamma globulin sera were prepared in rabbits and conjugated with fluoresceine isothiocyanate in the laboratory. Free fluoresceine was removed by passage through a Sephadex column and absorption with tissue powder. One such conjugate with an optimum titre of 1 in 30 was used throughout the present study. Sera were tested in parallel with this conjugate diluted 1 in 30 in buffered saline and also diluted 1 in 30 with 0·04 per cent. Evans blue T 1824 (G. T. Gurr, catalogue number 122) in buffered saline; the diluted conjugates were applied for 30 min. at 35°C.

The sera examined were consecutive specimens sent to the laboratory for TPI tests. Tests were set up with each method on different days and the slides read by the same observer, the intensity of fluorescence being recorded as:

Reactive	(*a*)	++++	Very brilliant apple-green fluorescence.
	(*b*)	+++	Brilliant fluorescence.
	(*c*)	++	Definite fluorescence but less than (*b*).
Non-reactive	(*d*)	+	Faint fluorescence.
	(*e*)	±	Treponemes just visible.
	(*f*)	0	Treponemes not visible under ultra-violet light.

When no treponemes could be seen under ultra-violet light, their presence was confirmed by examination under visible light.

Positive and negative control sera were included in each batch of tests; the positive control was tested at dilutions known to give maximal (++++) and minimal (++) reactive results. The light source used was a 250 watt ME/D high-pressure mercury vapour lamp, light being passed through a 1 cm. trough of 5 per cent. copper sulphate to remove residual red light. A Chance-Watson 2 mm. OX7 exciter filter was used with a Watson OY 12 barrier filter on the eyepiece. A Zeiss 1/7 in. oil immersion objective N.A. 0·9 was used with a X4 Watson compensating eyepiece.

Results

220 sera were examined by the two FTA techniques. The results in terms of reactivity are shown in Table I (opposite).

When the results of the two methods were classed simply as "reactive" or "non-reactive", there was little to choose between them; the conjugate diluted with Evans blue gave 48·6 per cent. reactive results against 47·7 per cent. when it was diluted with buffer.

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TABLE I

COMPARISON OF REACTIVITY OF THE TWO FTA TECHNIQUES

TPI	No. of Sera	Conjugate in:	FTA	
			Reactive	Non-reactive
Positive	99	Buffer	93	6
		Evans blue . .	92	7
Doubtful	27	Buffer	7	20
		Evans blue . .	10	17
Negative	94	Buffer	5	89
		Evans blue . .	5	89

The results with individual sera are analysed in Tables II, III, and IV, which show that the readings of intensity of fluorescence were identical in 161 sera (73 per cent.) and varied by plus or minus one step in the scale in a further 47 (21·4 per cent.) and by more than one step in twelve (5·5 per cent.). In the TPI-positive sera the discrepancies showed that the conjugate diluted with Evans blue tended to give slightly more intense reactions than when it was diluted with buffered saline; the reverse was true of the discrepancies in the TPI-doubtful and TPI-negative sera.

TABLE II

COMPARISON OF INTENSITY OF FLUORESCENCE in 99 TPI-POSITIVE SERA

Buffer	Evans Blue					
	++++	+++	++	+	±	0
++++	5	1	1	—	—	—
+++	3	19	3	—	—	—
++	—	9	50	—	1	1
+	—	—	1	1	—	1
±	—	—	—	—	1	—
0	—	—	—	1	—	1

TABLE III

COMPARISON OF INTENSITY OF FLUORESCENCE IN 27 TPI-DOUBTFUL SERA

Buffer	Evans Blue					
	++++	+++	++	+	±	0
++++	1	—	—	—	—	—
+++	—	—	—	—	—	—
++	—	—	5	—	—	1
+	—	—	4	1	2	2
±	—	—	—	1	2	3
0	—	—	—	—	—	5

TABLE IV

COMPARISON OF INTENSITY OF FLUORESCENCE IN 94 TPI-NEGATIVE SERA

Buffer	Evans Blue					
	++++	+++	++	+	±	0
++++	—	—	—	—	—	—
+++	—	1 (*a*)	—	—	—	—
			—	—	—	—
++	—	—	2 (*b*) (*c*)	2 (*d*) (*e*)	—	—
+	—	—	1 (*f*)	2	3	3
±	—	—	1 (*g*)	2	2	8
0	—	—	—	—	4	63

Note	Reiter Protein Complement-Fixation Test	Wassermann Reaction Cardiolipin	Price's Precipitation Reaction	Clinical Diagnosis Supplied
(*a*)	—	—	—	Treated latent syphilis
(*b*)	++	++	Positive 1 in 4	Penile sore
(*c*)	++	++	—	Pregnant
(*d*)	++	—	—	Male West Indian aged 50 Optic atrophy
(*e*)	—	++	+neat serum	West Indian Pregnant
(*f*)	—	—	—	Reiter's syndrome
(*g*)	—	—	—	Female aged 70 Luetic aortitis

Conclusion

The reading of the FTA test depends on a subjective impression of the intensity of fluorescence. Quenching of background fluorescence and the staining of any debris present in the films by a contrasting colour has been found of material help in reading the results.

REFERENCES

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Le bleu "Evans" comme colorant de fond dans le test F.T.A.

RÉSUMÉ

Le résultat du test F.T.A. dépend d'une impression personnelle de l'intensité de la fluorescence. Si la fluorescence du fond est éteinte et les débris qui se trouvent sur le film sont colorés avec un colorant qui fait contraste (dit bleu Evans), les résultats deviennent plus clairs.